Native O-Glycoprotease, Mass Spec Grade

Cat. No. O-Glycoprotease-46 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	
Description	O-Glycoprotease is an immunoglobulin-degrading metalloprotease (IMPa) secreted by Pseudomonas aeruginosa. It specifically cleaves the peptide bond on the N-terminal side of serine (S) or threonine (T) residues in O-glycosylated glycoproteins or glycopeptides. It shows no preference between mucin-type Olinked glycans with or without sialic acid. This enzyme is used for characterizing O-glycosylated proteins and analyzing O-glycan structures of glycoproteins.
Form	Solution containing 20 mM Tris-HCl (pH 7.8 at 25 centigrade), 100 mM NaCl.
Bio-activity	50,000 units/mg
Molecular Mass	97 kDa
Purity	Determined by liquid chromatography at 280 nm by measuring impurity protein peak intensity. The chromatographic peak intensity of O-glycoprotease is greater than 99.0 %.
Storage	Store at -20 centigrade (freezer).
Concentration	1 μg/μL
Shelf life	24 months at -20 centigrade

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Usage Notes	Digestion protocol: 1. Suitable digestion buffer: 20 mM Tris or HEPES buffer with pH maintained between 7 and 8. 2. Remove detergents from either native or denatured glycoprotein samples. Use a protein-to-enzyme mass ratio of 20:1, with protein concentration controlled at 0.2-0.5 μg/μL. Incubate in a 37 centigrade dry bath for at least 2 hours.
Specificity	Using bovine serum fetuin as substrate (denatured digestion), followed by ZIC-HILIC enrichment and ESI-MS/MS mass spectrometry analysis, the enzyme showed >95 % specificity for O-glycosylated serine/threonine (S/T) residues.
Unit Definition	1 μg/μL O-glycoprotease, diluted 10-fold, reacted with 5 μmol of fetuin glycoprotein under the following conditions: 20 μL of 20 mM Tris buffer (pH 8.0), incubated in a 37 centigrade dry bath for 2 hours, analyzed by SDS-PAGE electrophoresis.
LC-MS/MS Analysis	20 μg Fetuin glycoprotein was denatured using BT reagent, reduced and alkylated, followed by UFD ultrafiltration desalting. Add 100 μL of 20 mM Tris buffer and 1 μL Oglycoprotease, incubate for 2 hours, enrich with a ZIC-HILIC tip column, and analyze with QE480 LC-MS to fully identify S/T site modifications and the composition of O-glycans.
pH Range	pH 7-8 for best activity.
Sensitivity	O-Glycoprotease is sensitive to SDS and other detergents; buffer pH must be strictly maintained between 7 and 8 to avoid loss of activity.

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